IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of

Hisashi NAGAMOTO et al.

Group Art Unit: 1654

Serial No.: 10/566,214

Examiner: Anna Pagonakis

Filed: January 27, 2006

For: CARBOSTYRIL DERIVATIVES FOR ACCELERATING SALIVATION

DECLARATION

Honorable Commissioner for
Patents and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Hisashi NAGAMOTO, a citizen of Japan residing at 55-25, Nakagirai-Aza-Inamoto, Matsushige-cho, Itano-gun, Tokushimaken, Japan, declare and say that:

1. I was graduated from Hokkaido University, Faculty of Science in March 1979, and completed the course of Doctor at the same university, Graduate School of Science in March 1984.

Since April 1984 up till the present, I have been an employee of Otsuka Pharmaceutical Co., Ltd. I have a technical background in biological and pharmaceutical fields, particularly in biochemistry and pharmacology, and has been engaged in research and development of a plenty of medicaments at the Research Institute of said company.

2. I am one of the inventors for the invention described in U.S.

Patent Application No. 10/566,214 (hereinafter, referred to as "the present invention") and am familiar with the subject matter thereof.

3. I have read the cited Urashima et al., WO 97/13515 and am familiar with the subject matter thereof.

5. Under my direction, the following comparative experiments have been done.

I. Experiment 1

Muscarine M3 receptor (CHRM3) agonist assay

1) Test materials:

(i) Compound of the present invention: 2-(4-chlorobenzoyl-amino)-3-(2-quinolon-4-yl]propionic acid (general name: Rebamipide)

(ii) Acetylcholine, as a positive control (it is known that acetylcholine has various pharmacological activities, among which it has also muscarine activity (e.g. miotic action, an activity of increasing salivary secretion).

(iii) Pilocarpine: as a positive control (it is known that pilocarpine is a parasympatetic agonist and is useful as a miotic agent for the treatment or diagnosis of glaucoma, and has also other activities such as increased salivary secretion, increased acid secretion.

(iv) Phosphate buffered saline (PBS), as a negative control

2) Test method:

Into Xenopus oocyte was inserted cRNA (rat or human origin) of muscarine M3 receptor (CHRM3) to express muscarine M3 receptor (rat or human origin) on the cell membrane. The resulting cells were

stimulated with 0.1μM, 1μM and 10μM of rebamipide, and with 10μM of acetylcholine and 0.1μM and 1μM of pilocarpine, and the electrophysiological response (change of electric current, μA) due to the stimulation with rebamipide and the control materials, that is, due to signaling of rat and human muscarine M3 receptor (CHRM3), was measured by electrophysiological measuring system (manufactured by Hitachi).

3) Test results:

The above test results are shown in the following Table 1 (stimulation with rat muscarine M3 receptor (CHRM3)) and Table 2 (stimulation with human muscarine M3 receptor (CHRM3)).

Table 1

(Stimulation with rat muscarine M3 receptor (CHRM3))

Test Materials	Rate of response*1	Change of Average electric current (μA)
Controls:		
PBS (negative control)	0/6 (0%)	ND*2
Acetylcholine (10μM, posit. contl.)	6/6 (100%)	3.79±0.99
Pilocarpine (0.1μM, posit. contl.)	5/5 (100%)	0.78±0.12
Pilocarpine (1μM, posit. contl.)	5/5 (100%)	1.69±0.59
Test compound:		
Rebamipide (0.1μM)	0/6 (0%)	ND*2
Rebamipide (1μM)	0/6 (0%)	ND*2
Rebamipide (10μM)	0/6 (0%)	ND*2

[Note] *1: Times of response/total test times (% of response)

*2: ND = not detected (<0.2μA)

Table 2

(Stimulation with human muscarine M3 receptor (CHRM3))

Test Materials	Rate of responding*1	Change of Average electric current (μA)
Controls:		
PBS (negative control)	0/6 (0%)	ND*2
Acetylcholine (10μM, posit. contl.)	5/6 (83.3%)	2.02±0.30
Pilocarpine (0.1μM, posit. contl.)	3/5 (60%)	0.48±0.15
Pilocarpine (1μM, posit. contl.)	5/5 (100%)	1.10±0.32
Test compound:		
Rebamipide (0.1μM)	0/5 (0%)	ND*2
Rebamipide (1μM)	0/5 (0%)	ND*2
Rebamipide (10μM)	0/5 (0%)	ND*2

[Note] *1: Times of response/total test times (% of response)

*2: ND = not detected (<0.2μA)

As is shown in the above test results, the electrophysiological response was observed in all or most test times in case of stimulation with the positive control materials, acetylcholine and pilocarpine, but on the other hand, any response was not observed by stimulation with the test compound rebamipide like in negative control (with PBS). Thus, the with respect to the signaling of rat and human muscarine M3 receptor (CHRM3), a change of electric currency was observed by the stimulation with 10μM of acetylcholine and 0.1μM and 1μM of pilocarpine, but on the contrary, no significant change of electric currency was observed with 0.1μM, 1μM and 10μM of rebamipide. Accordingly, it was confirmed that the compound of the present invention is very weak in the activity mediated by a muscarine M3 receptor (CHRM3).

II. Experiment 2

Test of promoting secretion in lacrimal gland

1) Test material:

Compound of the present invention: 2-(4-chlorobenzoyl-amino)-3-(2-quinolon-4-yl)propionic acid (general name: Rebamipide)

2) Test method:

A suspension of Rebamipide in 5% carboxymethyl cellulose (CMC) or the vehicle (5% CMC) (each 2.5 mL/kg) were orally administered with a disposable tube (for rat, manufactured by Futigami Kikai, Japan) and a syringe (1 mL Termo Syringe®, manufactured by Termo Corporation, Japan) to male rats (7 weeks old, each group, 10 rats). Rebamipide was administered in a dose of 30 mg/kg and 100 mg/kg. At 2 hours and 4 hours after administering Rebamipide or the vehicle (5% CMC), the tear volumes from both eyes were taken with a Schirmer strip (1.5 mm x 15 mm, manufactured by Showa Yakuhin Kako K.K.), wherein the top of the Schirmer strip was inserted into the lower eyelid, and after one minutes, the strip was taken out, and the length of wet strip was read on a scale. The tear volume was shown in the wet length (mm) of Schirmer strip in average of both eyes.

3) Test results:

The results are shown in the following Table 3.

Table 3

Test Group	Schirmer score (mm) (Mean±SE)	
	After 2 hours	After 4 hours
Control (0.5 % CMC)	7.7±0.5	7.0±0.6
Rebamipide (30 mg/kg)	7.6±0.3	7.4±0.3
Rebamipide (100 mg/kg)	8.0±0.7	7.8±0.7

As is clear from the above test results, when the compound of the present invention rebamipide was orally administered to rats, the tear volume was almost the same as in the control rats administered with the vehicle. This means that the compound of the present invention, rebamipide has almost no effect on the promotion of secretion in lacrimal gland when administered orally.

III. Discussion

As is clear from the above-mentioned Experiment 1, the compound of the present invention has no activity mediated by a muscarine M3 receptor (CHRM3). It is known that cevimeline hydrochloride, being a muscarine receptor agonist, is used as a salivation accelerators, but it has some defects, for example, the possible side effects in digestive system, such as nausea, vomiting, anorexia, abdominal discomfort, stomachache. The compound of the present invention has no activity mediated by a muscarine M3 receptor contrary to the conventional salivation accelerators, and is known to be useful also as an anti-ulcer agent (cf. JP-B-63-35623), an agent for treatment of gastric inflammation (cf. JP-A-3-74329) and an agent for

protecting intestinal mucosa disorder (cf. JP-A-6-211662) with much less sides effects in digestive system, such as nausea, vomiting, anorexia, abdominal discomfort, stomachache. Thus, the compound of the present invention is useful for the acceleration of salivation secretion without undesirable side effects.

Further as is clear from Experiment 2, when administered orally, the compound of the present invention shows less promoting secretion in lacrimal gland, which means that the compound of the present invention is not effective for tear secretion by oral administration. The excellent effects of the compound of the present invention on the acceleration of salivation by oral administration have been well supported by the animal test and clinical tests (cf. the present description pages 19 to 25, EXPERIMENT 2, 3 and 4). The compound of the present invention has characteristics that it is more potent effects for acceleration of salivation or for prophylaxis or treatment of xerostomia by oral administration in comparison with the effect for promoting secretion in lacrimal gland with less side effects such as no activity mediated by muscarine M3 receptor as well as less side effects on digestive tract. Such characteristic feature of the present invention is not taught or even suggested by the cited Urashima et al., WO 97/13515.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 19 day of March, 2009.

Hisashi Nagamoto